Wu Sheng, H., L. Ryvarden, et al. (1997). ***Antrodia camphorata*** **("niu-chang-chih"), new combination of a medicinal fungus in Taiwan.** **Botanical Bulletin of Academia Sinica** **38(4): 273-275.**
A new combination, ***Antrodia camphorata*** (M. Zang & C.H. Su) Shena H. Wu, Ryvarden & T.T. Chang, is proposed for *Ganoderma comphoratum* M. Zang & C.H. Su, a name originally based on a polypore with contaminating *Ganoderma* spores. *Antrodia cinnamomea* T.T. Chang & W.N. Chou is reduced to a taxonomic synonym of *A. camphorata*. The species is famous and highly valued in Taiwan as a medicine, and is restricted to a Taiwanese endemic tree species, Cinnamomum kanehirai.

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Antioxidant properties of *Antrodia camphorata*

Ling-Chuan Huang , Jeng-Leun Mau , Shih-Jeng Huang , Chin-Chu Chen ,

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Abstract

Fresh fruit bodies of *Antrodia camphorata* contained only 31.16% of dry matter and 31~37% crude fat. Carbohydrate and fiber were also major components, comprising 54~59% of the dry matter. In mycelia, the fiber only comprised 7.21% of the dry matter. Besides the high carbohydrate content, ash and protein contents were higher in mycelia. However, reducing sugars were predominantly components in mycelial carbohydrates. Methanol extract of air-dried mycelia and fresh fruit bodies showed an excellent antioxidant activity, whereas that of air-dried fruit bodies was less effective.

Related Articles

Ling-Chuan Huang, Jeng-Leun Mau: Department of Food Science, National Chung-Hsing University, Taichung 402, Taiwan, ROC **Shih-Jeng Huang, Chin-Chu Chen**: Biotechnology Center, Grape King Inc., Chungli 320, Taiwan, ROC

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New type of cancer-fighting fungus developed

Published:February 13, 2003
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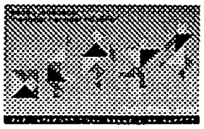
Biotechnology researchers at the Southern Taiwan University of Technology, in cooperation with a local biotechnology company, have developed what they described as an improved form of a potent cancer-fighting type of mushroom fungus called **Antrodia camphorata**.

The product has been shown to suppress cancer cells in mice. The two university and biotechnology company on Wednesday jointly announced that they have successfully developed a method to culture **Antrodia camphorata** into a solid form. In the future, the product will be packaged and marketed, becoming another instrument for those stricken with cancer to fight the disease.

The university's president, Chang Hsin-hsiung, on Wednesday said that the university's research team has successfully developed an anti-cancer agent from **Antrodia camphorata** in cooperation with the local company. **Antrodia camphorata** is unique to Taiwan, and the bodies have applied for patents in a number of countries to protect their discovery. Chang said clinical tests on cells and animals have proven successful.

Antrodia camphorata is a unique mushroom fungus used for medicinal purposes. It is only found in a type of camphor tree that is specially protected on Taiwan. **Antrodia camphorata** has been used in the private sector here with positive results.

Since the type of camphor tree here is a protected plant, the amount of **Antrodia camphorata** found here in the wild is limited. The market price for a catty is nearly NT$100,000 (US$2,880). Most **Antrodia camphorata** products found on the market at present are derived from liquid **culturing**. The effective ingredient in this type, however, pales considerably when compared with the solid cultured



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The two bodies have adopted special techniques in the solid **culturing** of the item, which increases its quality. After two years of research, the cooperative team made a major breakthrough when precision instruments indicated that the amount of triterpenoids in their product was substantially higher than that of products already on the market. Clinical experiments on cells and animals confirmed the cancer-fighting property of the product and that the potency of it was similar to that found in the wild.

The product was found to effective in fighting lung cancer, breast cancer, liver cancer and uterine cancer, with success seen in up to 80 percent of animal trials. Tests are presently being carried out on mice to determine the effectiveness in fighting intestinal cancer and heart cancer.

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SUGAR FLUX IN RESPONSE TO CARBOHYDRATE- FEEDING OF CULTUREI *ANTRODIA CAMPHORATA*, A RECENTLY DESCRIBED MEDICINAL FUNGUS IN TAIWAN

I-Hung Lee [1], Chi- Ting Chen [2], Hsiao- Chuan Chen [2], Wen- Chi Hsu and Mei- Kuang Lu [2]

[1] *China Medical College, Taichung, Taiwan*

[2] *National Research Institute of Chinese Medicine, Taipei, Taiwan*

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Antrodia camphorata, a wood rooting and traditional Chinese medicinal fungus, grew on a range of carbohydrates including glucose, fructose, xylose, rhamnose, maltose, galactose and sucrose. The effects of carbohydrate feeding in the growth medium of *A. camphorata* were examined both in the respects of growth rate and the monosaccharide composition in the free sugar pool of the cells. Both glucose and galactose as the carbon source greatly enhanced growth rate in the concentration of 40 g L^{-1}. Mycelial growth was insensitive to the addition of rhamnose, xylose,

and maltose in all tested concentrations (10 – 80 g L^{-1}). Analysis of 60- day- old mycelia showed glucose induced the formation of free arabitol and glucose. Sucrose enhanced the mannitol synthesis. The addition of rhamnose was able to up- regulate the synthesis of rhamnose in the cells. Galactose up- regulated the formation of myo- inositol and glucose. Xylose down- regulated the synthesis of glucose and mannitol.

Key words: ***Antrodia camphorata***, Fungi, Sugar analysis, HPLC, Carbohydrates.

INTRODUCTION

Antrodia camphorata (Basidiomycetes) is a medicinal fungus with limited distribution in Taiwan which prohibits the collection of sufficient quantities for extensive use as adrug remedy. It has been used for anticancer, antidots, and antichromic materials. Tissue and cell culture of this species for the mass- produceion

Correspondence to: Dr. Mei-Kuang Lu, National Research Institute of Chinese Medicine, TEL:886-2-28201999*7391, FAX:886-2-28264276, E-mail: mklu@cma23.nricm.edu.tw

pharmaceutical products is the aim of our laboratory activities. Chemical compounds found in *A. camphorata* included sesquiterpene lactone, steroids and triterpenoids [1-6] among which triterpenoids have ant and antiserotonergic activities. [5] Until now, no report was found on the description of the carbohydrate c of *A. camphorata.*

Interest in this study lies in the possibility of developing specific methods for detection and identification of carbohydrates of this species. The present investigation of carbohydrates of *A. camphorata* mycelia will facilitate understanding of the sugar flux of fungi in general. We reported here on the carbohydrate compositio *camphorata*. Quantification of the carbohydrates and profile analysis was carried out by anion exchange chromatography with pulsed amperometric detection for its sensitive analysis of carbohydrates in HPLC

system. [7] The carbohydrate contents of mycelia from several carbohydrate- feeding in the culture medium wei determined and compared.

MATERIALS AND METHODS

A. camphorata strains

The isolation of *A. camphorata* accession number 35396 was obtained from Culture Collection and Research Center (CCRC) [8]. *A. camphorata* was cultivated on potato- dextrose-agar 39 g L $^{-1}$ 20 g L $^{-1}$, enriched with the corresponding amounts of carbohydrates in the medium, pH 5.6, 28°C under dark conditions. Growth rate of the culture was determined by measuring the radius of each colony. Growth media used in the solid culture were purchased from Sigma Co. (Saint Louis, MO, USA). LC grade organic solvents were purchased from E. Merck Co.

Sample preparation

For isolation of the free monosaccharides, as well as the hydrolysis of the polysaccharide fractions, 60-day- old cultures were used. In the end of the incubation, mycelia were rapidly washed under an aspirator-suction with 1L 250 mM NaCl, lyophilyzed, and stored at 4°C for determination of carbohydrate composition. The mycelia were ground into powder and extracted with hot 80% (v/v) ethanol. [9] The aque collected and evaporated to remove acid residues. The dried pellet was suspended in Q- water and passed through a Millipore- GX nylon membrane.

Chemical analysis of neutral sugars

Analyses of neutral sugars obtained from cell- lysates prepared with hot ethanol extraction were carried c by high- performance anion- exchange chromatography (HPAEC) system (Dionex, USA) equipped with a gradient pump, a pulsed amperometric detector (PAD- II), and an anion- exchange column (Carbopac PA-10,

4.6 × 250 mm). Samples were applied using a autosampler (AS3500, SpectraSYSTEM ™) v valve with a 200 ∝l sample loop. The effluent was mixed with 0.3 M NaOH at the rate of 0.3 ml min maintain the basicity required for the detector sensitivity. Area under the curves were integrated with an AI- 45 (Dionex). Identification and quantification of carbohydrates were made in comparison with authentic standards and calibration curve of the standard reference peaks. Carbohydrate standards were obtained from Sigma Co. (Saint Louis, MO, USA). Conditions of separation were performed at 90 mM NaOH, 1ml min 15-min column wash with 200mM NaOH followed by a 15- min equilibration.

Hydrolysis of EtOH- insoluble residues

EtOH- insoluble residues were prepared as follows: a final concentration of 80% (v/v) EtOH was added t the dried mycelia and heated under 80 °C for 20 min. After cooling, the tube was centrifuged (3000 g, 5 min), the supernatant removed, replaced by 80% (v/v) aqueous EtOH, and heated at 80 °C 20 min. Heating was done twice and then washed. The residue was dried and ground. EtOH- insoluble residues were hydrolyzed under th condition of 6N HCl, 6 h, 80°C. The acid- hydrolysates were evaporated at 40 °C to remove acid residues. The dried pellet was suspended in Q- water and passed through a Millipore- GX nylon membrane before HPLC analysis.

Data collection

The value of two independent observations were averaged as presented experimental data. Statistically significant comparisons: *t*- test $P < 0.05$.

RESULTS

Effect of carbohydrate feeding on mycelia growth

Growth rate of *A. camphorata* mycelia was determined by measuring the growth radius of the colony on solid medium (Figure 1). In general, the growth curve showed that lag phase took place in the first four days incubation, and log phase extended from day five to day 20. Comparisons were made between control and various sugar- feedings. The addition of carbohydrates in the medium enhanced mycelial growth for almost all the tested sugars in all the applied dosages. At the 56 th days of incubation, it showed thatthe additio

in the medium led to the observation that the relative rate of growth was 16, 26.4, 45 and 26 for the applied dosage of 10, 20, 40, and 80 g L $^{-1}$, respectively. For the addition of galactose in the medium led to t observation that the relative rate of growth was 31, 25.4, 47, 29.4 for the applied dosage of 10, 20, 40, and 80 g L $^{-1}$, respectively. Among them, 40 g L $^{-1}$ was the most effective dosage for the glucose or galactose- fee the medium. Mycelia growth was insensitive to the feeding dosage for the carbon source of rhamnose, xylose,

Figure 1. Effects of the addition of various carbohydrates on the growth of *A. camphorata. A. camphorata* was incubated without (control) or with the addition of (A) glucose; (B) xylose; (C) rhamnose; (D) galactose; (E) fructose; (F sucrose; (G) maltose at the indicating dosage. Each point was the average of two independent observations.

Figure 2. Anion- exchange chromatogram with pulsed amperometric detection of seven standards of monosaccharides. Conditions for HPLC were described as materials and Methods. Detection was by pulsed amperometric detection, 30 nC scale. The identity of monosaccharide was as follows: 1, myo- inositol; 2, arabitol; 3, dulcitol; 4, mannitol; 5, fucose;6, rhamnose;7, glucose.

and maltose. At the 56 th days of incubation, it showed that the addition of sucrose and maltose in the medi to the observation that the relative rate of growth was 33, and 23 for the applied dosage of 80 g L

Effect of carbohydrate feeding on free sugar biosynthesis of the mycelial cells

Anion- exchanged chromatography followed by pulsed amperometric detection was used to determine th free sugars in the cells. Using 90 mM NaOH as a column effluent, myo- inositol, arabitol, dulcitol, mannitol, fucose, rhamnose and glucose were readily separated in 8 min at a flow rate of 1ml min $^{-1}$ (F

Free sugars were extracted from the cells by hot ethanol (80%). Comparisons were made between differe carbohydrates source in the medium in combination with different dosage of the carbohydrates (Figure 3). The overall detected carbohydrates were on a scale of less than 0.1% of dry weight. Without the addition of carbohydrate in the PDA and malt basal medium (control), arabitol and mannitol were the major free sugars in the mycelium. The amount of arabitol and mannitol in the mycelium were in the concentration of 1.70 and 1.68 mg g $^{-1}$ dry weight, respectively. With the addition of glucose in the basal medium, arabitol and glucose were a direct increase with the increase of glucose concentrations in the medium. The amount of arabitol were 2.62, 4.95, and 5.96 mg g $^{-1}$ dry weight for the glucose- feeding at the concentration of 10, 40, and 80 g L respectively. The amount of glucose were 1.1, 1.1, and 3.8 mg g $^{-1}$ dry weight for the glucose- concentration of 10, 40, and 80 g L $^{-1}$, respectively. Xylose seemed to be a negative factor in the format mannitol and glucose by the fact that it was a negative dosage effect. The amount of mannitol were 1.2, 0.64, and 0.43 mg g $^{-1}$ dry weight for the xylose- feeding at the concentration of 10, 20, and 40 g L

Figure 3. Compositional analysis of monosaccharide for the *A. camphorata* incubated with (A) glucose; (B) xylose; (C) rhamnose; (D) galactose; (E) fructose; (F) sucrose; (G) maltose; (H) control at the corresponding concentrations for 60 days. Collected data were the average values of two analyses.

amount of glucose were 0.45, 0.27, 0.21 and 0.20 mg g $^{-1}$ dry weight for the xylose- feeding at the c of 10, 20, 40, and 80 g L $^{-1}$, respectively. The addition of rhamnose, rhamnose was detected in the mycelia was not detected in other treatment, and was in a direct response. The amount of rhamnose were0.3, 0.6, 4.0 an 5.4 mg g $^{-1}$ dry weight for the rhamnose- feeding at the concentration of 10, 20, 40, and 80 g L The addition of galactose was able to induce the formation of glucose in the concentration of 40 g L High concentration of galactose (80 g L $^{-1}$) in the medium would inhibit the synthesis of glucose. The a glucose were 0.2, 0.3, 2.4, and 1.1 mg g $^{-1}$ dry weight for the galactose- feeding at the concentration of : and 80 g L $^{-1}$, respectively. For the synthesis of myo- inositol, it was a direct response to galactose. The amou of myo- inositol were 0.06, 0.06, 0.07, and 1.00 mg g $^{-1}$ dry weight for the galactose- feeding at the c of 10, 20, 40, and 80 g L $^{-1}$, respectively. Fructose was able to induce the synthesis of mannitol when fruc concentration was less than and equal to 20 g L $^{-1}$. The amount of mannitol were 4.1 and 7.1 mg g

the fructose- feeding at the concentration of 10, and 20 g L^{-1}, respectively. With the addition of sı basal medium, mannitol were increased with the direct dosage of sucrose. The amount of mannitol were 0.7, 2. 3.6 and 7.8 mg g^{-1} dry weight for the sucrose- feeding at the concentration of 10, 20, 40, and 80 g L respectively. With the addition of maltose in the medium, mannose, and glucose were increased when the maltose concentration was less than and equal to 40 g L^{-1}. High concentration of maltose could gr the synthesis of glucose. The amount of glucose were 1.00, 4.50, and 0.01 mg g^{-1} dry weigh feeding at the concentration of 10, 40, and 80 g L^{-1}, respectively.

In order to examine the feeding of carbohydrates on the effect of the sugar composition in the non- free s pool, we analyzed the monosaccharide composition of EtOH- insoluble portion. Comparisons were made between glucose and xylose- feeding mycelia (Figure 4). The EtOH- insoluble residues could be regarded as preparations of unlignified cell walls.[10] The cell wall hydrolysates (CWH) neutral sugar fraction was made up of glucose. This is also true for the reports of Burczyk *et al.*[11] Feeding of glucose grea amount of glucose both in the CWH portion and in the free carbohydrate pool. The maximum presence of glucose in the CWH was occurred when feeding of glucose at the concentration of 20 g L^{-1}. of feeding, the glucose tended to accumulate in the free- sugar pool than in the CWH portion. The amount of glucose in the CWH portion were 2.1, 9.2, 10.5, 9.3 and 8.1 mg g^{-1} dry weight for the glucose- concentration of 0, 10, 20, 40 and 80 g L^{-1}, respectively. The amount of glucose in the free sugar port 0.2, 1.1, 0.4, 1.0 and 3.8 mg g^{-1} dry weight for the glucose- feeding at the concentration of 0, 10, 20, 40 a L^{-1}, respectively. Feeding of xylose in the culture medium seemed to inhibit the accumulation of free glucose. No parallel change of glucose with the feeding of xylose was observed in the CWH portion. The amount of glucose in the CWH portion were 2.1, 7.5, 3.7, 8.9 and 6.0 mg g^{-1} dry weight for the xylose- f concentration of 0, 10, 20, 40 and 80 g L^{-1}, respectively. The amount of glucose in the free sugar porti

Figure 4. Compositional analysi hydrolysates and fi of the mycelia whic with glucose and xy corresponding dos hydrolysates was p insoluble fraction o extracts hydrolyzed 80°C 6 - 8 h. The fr was extracted and described in the m methods. Each valu of two independent

0.2, 0.4, 0.3, 0.2 and 0.2 mg g^{-1} dry weight for the xylose- feeding at the concentration of 0, 10, 20, 40 a L^{-1}, respectively.

DISCUSSION

In vitro culture of filamentous fungi, carbohydrate is a major nutrition to support the mycelial growth, rather than proteins which is the major source for bacterial **culturing**. In the search for optimal carbohydrate source and concentrations supplemented in the medium, we tested several neutral sugars in the combination with different feeding concentrations on the rate of mycelia growth (Figure 1). The addition of carbohydrates in the medium enhanced mycelial growth for almost all the tested sugars in all the applied dosages in this study. The results showed that glucose and galactose were the most effective carbohydrate source in the stimulating of mycelial growth. It suggested an important role of the configuration of carbon 2 and 3 on the mycelial growth.

Among the di- saccharides we tested, sucrose (glucose- α(1?2)- fructose) was efficient in inducing mycelia growth than maltose (glucose- α(1?4)- glucose).

In the examination of EtOH- insoluble portion (Figure 4), we hydrolyzed the intact carbohydrate compounds which may include polysaccharides, glycoproteins, lipopolysaccharides, and other macromolecules

with carbohydrate moity. The results showed that the intact glucose was at least eight to thirty times more than the glucose in the free form. Among the glycoconjugates in the mycelium, polysaccharides are suggested to be potentially useful, biologically active ingredients for pharmaceutical uses due to a variety of biological activitie such as mitogenic activity and activation of alternative pathway complement (APC) and plasma clotting activity [12]. Numerous mushroom polysaccharide fractions have been documented for their tumor inhibition activity, such as a glucan- protein complex from *Ganoderma tsugae* [13], lentinan (a 1?3 linked from *Lentinus edodes* [14], and schizophyllan from *Schizophyllum commune* [15].

In summary, the carbohydrate profiles of *A. camphorata* was established in a manipulated cellular syster The present analytical methodology is adequate for investigation of important cellular process. The report reveals that the sugar patterns in *A. camphorata* will provide useful information for further study on the possibl pathway in carbohydrate metabolism. The identification and characterization of polysaccharides of *A. camphorata* is in progress.

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藥用真菌，牛樟芝(*Antrodia camphorata*)，生長於不同含醣培養基下，醣類化合物合成的探討

李一宏[1] 陳其婷[2] 陳曉娟[2] 許文綺[2] 盧美光[2]

[1]中國醫藥學院 中國醫學研究所

台中

[2]國立中國醫藥研究所

台北

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牛樟芝，為一種近來在台灣被用作藥用真菌的木材腐生菌。本實驗將牛樟芝培養於含有不同濃度的葡萄糖、果糖、木醛糖、鼠李糖、麥芽糖、半乳糖及蔗醣培養基，以瞭解這些添加的醣類對於牛樟芝的生長速度及牛樟芝細胞內游離的單糖之影響。而發現 1.以葡萄糖及半乳糖在添加濃度 40 g L^{-1} 為碳源的培養基下，牛樟芝的生長速度快速。2.牛樟芝對於添加以鼠李糖、木醛糖、及半乳糖在 10- 80 g L^{-1} 的濃度測試範圍下，其生長速度無明顯的影響。在:長 60 天的牛樟芝菌絲体內，培養基添加葡萄糖能促進葡萄糖及阿拉伯糖的生成，蔗糖能促進甘露醇的生成，鼠李糖能促進細胞內鼠李糖的生成，半乳糖能促進葡萄糖及肌醇的生成，木醛糖的添加則抑制葡萄糖及甘露醇的生成。上述結果，將有助於牛樟芝菌絲体內碳水化合物代謝途徑作進一步的探討。

關鍵詞：牛樟芝，藥用真菌，糖類，HPLC，碳水化合物。

聯絡人：盧美光，國立中國醫藥研究所，台北市北投石牌立農街二段 155-1 號，TEL: 28201999 ext.7391。

Non-volatile taste components of Agaricus blazei, Antrodia camphorata and Cordyceps militaris mycelia

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Hui-Ling Changa, Guei-Rung Chaoa, Chin-Chu Chenb and Jeng-Leun Mau.

Department of Food Science, National Chung-Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan, ROC Biotechnology Center, Grape King Inc., Chungli 320, Taiwan, ROC

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Three non-Ganoderma medicinal mushrooms are currently popular in Taiwan, including Brazilian mushroom (Agaricus blazei), chang-chih (Antrodia camphorata) and northern caterpillar fungus (Cordyceps militaris). The moisture contents of three dry mycelia ranged widely from 6.65 to 14.91%. All mycelia were high in carbohydrate content with chang-chih being the highest. The protein contents ranged from 9.49 to 29.1%. Soluble sugars found were arabitol, glucose and trehalose, and the contents exceeded 10%. Total free amino acid contents ranged from 7.01 to 11.1 mg g-1 dry weight. Contents of monosodium glutamate-like components were relatively low and similar in Brazilian mushroom and chang-chih, but high in northern Cordyceps. Contents of bitter components were significantly high in Brazilian mushroom and northern Cordyceps. Contents of flavour 5'-nucleotides were similarly high in chang-chih and northern Cordyceps, and low in Brazilian mushroom. The three mushroom mycelia had different proximate compositions. However, northern Cordyceps and chang-chih might exhibit similar umami and sweet tastes.

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